

Colonization, hatching dates, and growth rates of juvenile *Hyporhamphus picarti* (Hemiramphidae), in the Nador lagoon (NE Morocco)

by

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Abstract. – The Nador lagoon, located in the northeastern part of the Moroccan Mediterranean coast, was sampled with a beach seine at six stations during different seasons, from 2012 to 2014. The African halfbeak *Hyporhamphus picarti* was collected among the fish species and some aspect of its biology and ecology (colonization of the lagoon, spawning period and growth) was described for the first time. This species was found only in late summer and autumn, and all the individuals captured were juveniles, indicating that the lagoon serves as an important nursery for this species. Growth increments in the *lapilli* otoliths were used to investigate its early life history. Assuming that the primary increments are deposited daily, age and hatching dates were determined by counting these increments in juvenile fish collected during late summer and autumn. The estimated ages of juveniles ranged from 39 to 58 days (TL: 68.10 ± 15.19 mm) in 2012, and from 42 to 77 days (TL: 101.03 ± 28.77 mm) in 2013. Juveniles collected in 2013 had significantly higher growth rates (2.35 mm. d⁻¹) than those collected in 2012 (1.88 mm. d⁻¹) regardless of temperature. For both years, the average otolith increments width increased until about the 25th day (corresponding to a size of about 28 mm TL), and then decreased. Back-calculated hatching dates of juveniles collected in the lagoon indicated that they hatched in summer, from June to September. These results indicate that *H. picarti* has a short spawning period during the warm period and uses the lagoon as a nursery ground in late summer and autumn.

Key words

Hemiramphidae
Hyporhamphus picarti
Mediterranean Sea
Nador lagoon
Otolithometry
Growth

Résumé. – Colonisation, dates d'éclosion et taux de croissance des juvéniles d'*Hyporhamphus picarti* (Hemiramphidae) de la lagune de Nador (NE Maroc).

La lagune de Nador, située dans la partie septentrionale de la côte méditerranéenne du Maroc, a été échantillonnée de 2012 à 2014 à l'aide d'une senne de plage au niveau de six stations et au cours de différentes saisons. Le demi-bec africain *Hyporhamphus picarti* figure parmi les espèces de poissons capturées et certains aspects de sa biologie et de son écologie (colonisation de la lagune, période de ponte et croissance) sont présentés pour la première fois. Cette espèce a été capturée seulement en fin d'été et en automne, et tous les individus étaient des juvéniles, ce qui indique que la lagune sert de nourricerie pour cette espèce. La microstructure des otolithes (*lapilli*) a été analysée pour étudier les premières phases de son cycle de vie. En assumant que les accroissements de l'otolithe sont déposés journalièrement, l'âge, la croissance et les dates d'éclosion ont été déterminés en dénombrant ces accroissements chez des individus capturés à la fin de l'été et en automne. Les âges estimés des juvéniles variaient de 39 à 58 jours (LT: 68.10 ± 15.19 mm) en 2012, et de 42 à 77 jours (LT: 101.03 ± 28.77 mm) en 2013. Les juvéniles capturés en 2013 avaient des taux de croissance significativement plus élevés (2.35 mm. j⁻¹) qu'en 2012 (1.88 mm. j⁻¹) mais sans relation avec la température du milieu. Pour les deux années, la largeur moyenne des accroissements de l'otolithe augmente jusqu'au 25^e jour (correspondant à une taille d'environ 28 mm LT), puis diminue. Les dates d'éclosion rétrocyclées à partir des juvéniles capturés dans la lagune ont indiqué que leur naissance se situe en été, de juin à septembre. Ces résultats indiquent que *H. picarti* a une période de ponte courte pendant la période chaude et que cette espèce utilise la lagune comme zone de nourrice-rie à la fin de l'été et en automne.

Coastal lagoons are among the most productive wetlands in the Mediterranean region (Crivelli and Ximenes, 1992; Franco *et al.*, 2012). They play an important ecological role in coastal ecosystems by providing a collection of habitat types for many species, and functioning as nursery areas and feeding grounds for marine estuarine opportunistic fish (Yáñez-Arancibia and Nugent, 1977; Clark, 1998). For

example, more than 199 fish species are present in coastal lagoons in the Atlantic-Mediterranean region (Pérez-Ruzafa *et al.*, 2010).

The Nador lagoon is the second largest lagoon complex in northern Africa (115 km²), and the broadest continental aquatic environment in Morocco. It is linked to the Mediterranean Sea only by the Boccana Channel. Its water

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depth ranges from 5 m in the north to 8 m in the south, and water salinity and temperature vary during the year from 39.5 to 43.5, and from 10°C (January) to 28°C (August), respectively (Lefebvre *et al.*, 1997; Ruiz *et al.*, 2006). The Nador lagoon ichthyofauna is not well-known (Bouchereau *et al.*, 2000), although the lagoon itself maintains a diverse fish community and supports important commercial fisheries (Kada *et al.*, 2009). For the first time in this location, the African halfbeak, *Hyporhamphus picarti* Valenciennes, 1847, is described.

Hemiramphidae, the halfbeaks, are one of the five families of the order Beloniformes inhabiting warm waters of the Atlantic, Indian, and Pacific Oceans (Collette, 2004). Most studies on the biology and ecology of halfbeaks have been done on species living either in Australia (Tibbetts and Carseldine, 2005; Stewart and Hughes, 2007; Fowler *et al.*, 2008; Earl *et al.*, 2011), or in Florida, on the west American coast (Berkeley and Houde, 1978; McBride and Thurman, 2003). Although *H. picarti* may play an important role in the lagoon fish assemblage, its biology and ecology have never been studied so far.

While lagoons support many essential fisheries, they are also among the most extensively modified and threatened aquatic environments (Pérez-Ruzafa *et al.*, 2010). For example, in the Ichkeul lagoon (Tunisia), *H. picarti* disappeared because of anthropogenic pressure (Sellami *et al.*, 2010). As many shallow coastal areas, the Nador lagoon functions as a nursery area for various fish species (Jaafour *et al.*, 2015). However, because the fishing activity is important in this

lagoon, by-catch of juvenile fish is high (Kada *et al.*, 2009). Like *H. picarti*, many juvenile fish that use the lagoon as nursery ground are commercially exploited species. From a conservational perspective and for sustainable management of fish species that use the lagoon as nursery ground, it is important to reduce the by-catch impact on juvenile fish. To study the importance of the Nador lagoon as a nursery ground for *H. picarti* and to describe aspects of its life history (colonization of the lagoon, spawning period, and growth), that may be useful for management and conservation, an experimental sampling over different habitats (e.g., plant covered ground, nude substrate, proximity of the artificial sea exit, or freshwater input) in the lagoon using a beach seine net was conducted at different seasons from 2012 to 2014. Otolith microstructures of juvenile *H. picarti* were analyzed to estimate growth rates and hatching dates, and to analyse their interannual variability in relation to temperature.

MATERIALS AND METHODS

Study area and sampling

The studied area of the Nador lagoon, also known as the “Sekha Bou-Areg” or “Mar Chica small sea”, is located in the northeastern part of the Moroccan Mediterranean coast (Fig. 1). The lagoon has an oval shape, is quite regular, and has a single inlet (the Bocana). The length of its major axis is about 23 km, while the minor axis is about 7 km. Its depth averages 4.8 m, and has a maximum of 8 m. Three water sources supply the lagoon: (a) the marine waters passing through the artificial inlet, which are always dominant; (b) the hydrogeological contributions from two aquifers – Gareb, located in southern Selouane, and Bou Areg, situated near the southern margin of the lagoon; and (c) the surface water inputs, with periodic flows of 10 small streams (“oueds”) (Ruiz *et al.*, 2006). In the lagoon, these different flows are oriented by internal clockwise currents, from the artificial inlet toward the NW coast, moving round the internal face of the sandy spit and the internal lagoon margin (Guélorget *et al.*, 1987). The bottom sediments of the continental margin consist mainly of black silts, whereas the rest of the lagoon is dominated by very fine to medium sands (Ruiz *et al.*, 2006). The macrophytes recorded in the lagoon correspond to the phanerogams, *Cyamodocea nodosa* and *Nanozostera noltii*, and the chlorobiont, *Caulerpa prolifera*. Generally, the invasive macroalga, *C. prolifera*, covers most of the bottom, except the central part, which is devoid of macrophytes, restricting the seaweed *C. nodosa* to small bands in the shallowest areas surrounding the lagoon (El Madani *et al.*, 2011).

Sampling was done at six stations between 08:00 and 18:00 on five visits in autumn (October 10th, 2012), winter (February 21st, 2013; and February 22nd, 2014), and in early

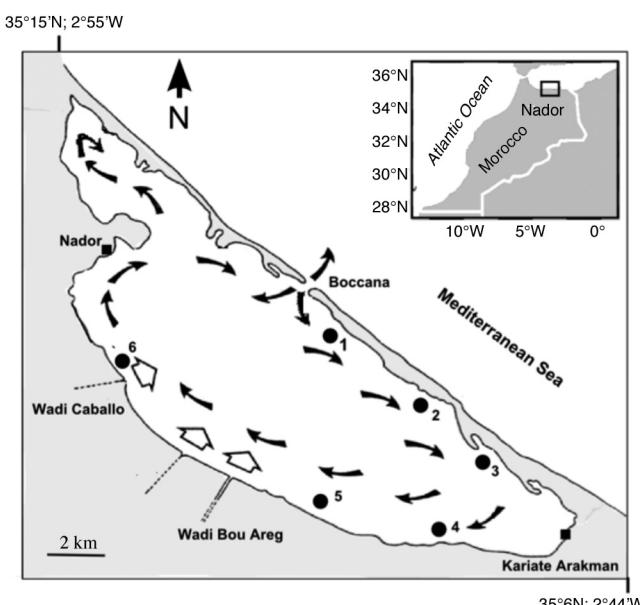


Figure 1. - The Nador lagoon and location of the six sampling stations. Black arrows indicate the hourly sense of the water flow in the lagoon. White arrows indicate the main freshwater inputs. Some geographical features cited in the text are indicated.

and late summer (June 26th, 2013; and September 7th, 2013), using a beach seine net (14 m length x 2 m height, with a mesh size of 3 mm). After each sampling, *H. picarti* individuals were collected and preserved in 95° alcohol, and the remaining fish were preserved in formalin. At the laboratory, all fish were identified to species level, counted, measured, and their abundance standardized as catch per unit effort (CPUE). *H. picarti* individuals were measured (total length TL, mm) to the nearest mm and weighed (total weight W, g) with a precision of 0.1 g.

Otoliths extraction, preparation, and analysis

Of the three pairs of otoliths, the *lapilli* were chosen for counting growth increments for the range of body lengths examined, since the sagittae required significantly more preparation to reveal or improve clarity of increments and were generally difficult to read. *Lapilli* were located with the use of polarized light, removed from the cranium, cleaned, air-dried, and mounted flat side up (i.e. convex side down) with cyanoacrylate glue on microscope slides. They were polished manually in the sagittal plane to the central primordial with a graded series of aluminium papers (3.0 to 0.1 μm), with periodic viewing to monitor progress until all the increments were visible.

Age, growth, and hatch-dates

Examination of otolith micro-increments was undertaken using a light microscope at 400X magnification, on which a high-resolution video camera was mounted, connected to a computer equipped with an image analyser developed for calcified structures (TNPC 5.0, NOESIS). The *lapillus* radius (OR, mm) was measured as the maximum distance from the core (nucleus) to the edge of the otolith. All increment counts were done along the anterior-posterior axis. Increments were counted twice by the same reader, and the mean of the two

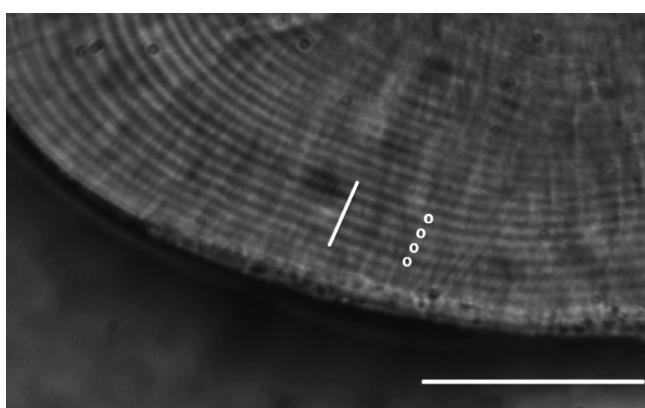


Figure 2. - Photomicrograph of a *lapillus* dissected from a juvenile *Hyporhamphus picarti* (TL = 75 mm) showing a growth increment sequence: white circles indicate daily growth increments and the white line a sequence of five growth increments used to measure the width of growth increments. Scale bar = 60 μm .

readings was considered as the specimen age in days; a third reading was made if the first two differed by > 5%. If the discrepancy persisted, the otolith was discarded. Among the 115 otoliths prepared, all were used in the analyses. Taking into account the narrow range of lengths analyzed, length-at-age data were calculated using the following formula:

$$\text{TL (mm)} = a + b \text{ age (days)},$$

where TL = the total fish length (mm); a = fish size at age 0; and b = instantaneous growth rate (mm d^{-1}).

Otolith incremental widths (μm) were measured (to the nearest 0.1 μm) every five increments from the innermost increment to the edge of the otolith (Fig. 2). The average growth rate of the otolith ($\mu\text{m/day}$) was then calculated by dividing its measured width by the number of daily increments counted (5). The hatching date distribution of juvenile *H. picarti* was back-calculated from age estimates and date of capture.

Water temperature was measured at each sampling date and during the two years of study, mean monthly air temperatures were obtained from the meteorological station of Nador. These temperatures are good proxy for the shallow water of the lagoon.

Statistical analysis

Statistics were performed with XLSTAT 2013 (Addinsoft TM). Differences in fish size and otolith increments width were analyzed using the parametric Student-t test. Growth curves, length-weight relationships, and otolith radius to fish-length relationships of individuals caught in 2012 and 2013, were compared using an analysis of covariance (ANCOVA).

RESULTS

Temperatures varied slightly during the two years of study. During winter 2012 (January, February and March), they were lower by 1 to 2°C compared to 2013, while they were higher in the 2012 summer (June, July and August) (Fig. 3).

Of the 14 fish species captured in the Nador lagoon, *H. picarti* was caught only during autumn 2012 (272 individuals) and late summer 2013 (29 individuals) samplings. All were juveniles (TL < 173 mm), and represented 35.23% and 5.67% of the total catch in 2012 and 2013, respectively.

In autumn 2012, 81 juveniles were caught, measuring between 38 and 127 mm TL, and weighing between 0.13 and 3.26 g. In late summer 2013, 29 juveniles were captured, measuring between 51 mm and 173 mm TL, and weighing between 0.11 and 8.43 g. The juvenile *H. picarti* collected in late summer 2013 were significantly bigger (101.03 ± 28.77 mm) than those collected in autumn 2012 (68.10 ± 15.19 mm) (t-test, $p < 0.05$; Fig. 4). There were significant

differences in the length-weight relationship of individuals caught in autumn 2012 and late summer 2013 (ANCOVA, $F = 2838.65$, $p < 0.0001$) (Fig. 5). The relations between otolith radius and fish total length were also significantly different between the two years (ANCOVA, $F = 171.64$, $p < 0.0001$) (Fig. 6). The fish in 2013 had a greater weight or otolith radius for a given length compared to 2012.

The estimated age of the juvenile caught varied between 39 and 58 days in 2012, and 42 to 76 days in 2013. For both years, the relationship between fish size (TL) and age (days) was described by the following linear equations (Fig. 7): $TL = 1.881 \times \text{age} - 19.29$ ($n = 52$; $r^2 = 0.63$; $p < 0.05$) in 2012 and $TL = 2.353 \times \text{age} - 35.57$ ($n = 29$; $r^2 = 0.71$; $p < 0.05$) in 2013. The instantaneous growth rates calculated were 1.88 mm.d^{-1} and 2.35 mm.d^{-1} in 2012 and 2013, respectively. Growth curves were significantly different between the two years (ANCOVA, $F = 154.80$; $p < 0.0001$).

Otolith increments width varied with fish age (Fig. 8). Mean increment widths ranged from 2.18 to $5.36 \mu\text{m}$ in 2012 and from 2.11 to $5.91 \mu\text{m}$ in 2013. A gradual increase in width was found until the 25th increment for both cohorts, but it decreased rapidly to reach those similarly measured near the nucleus. From age 25 to 40 days, increment

widths were significantly higher in 2013 compared to 2012 ($p < 0.05$). Hatching dates of the juveniles were back-calculated from the age and date of capture (Fig. 9). Juveniles collected in autumn 2012, hatched from August 11th to September 12th, 2012 (median hatching date was August 31st), and those collected in Autumn 2013, hatched from June 22nd to July 26th (median hatching date was July 6th).

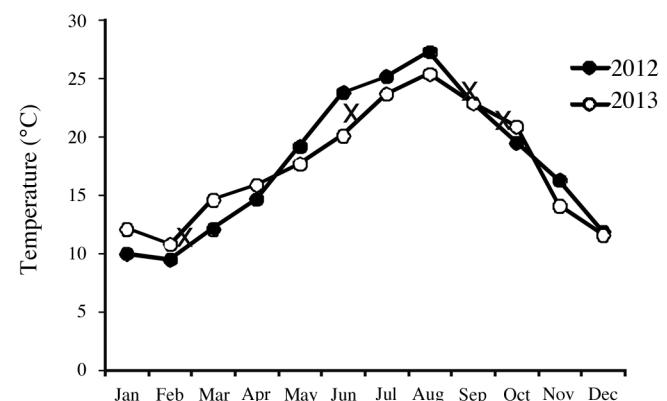


Figure 3. - Mean monthly air temperature ($^{\circ}\text{C}$) at Nador during 2012 and 2013. (X) indicate water temperature at each sampling date in 2012 and 2013.

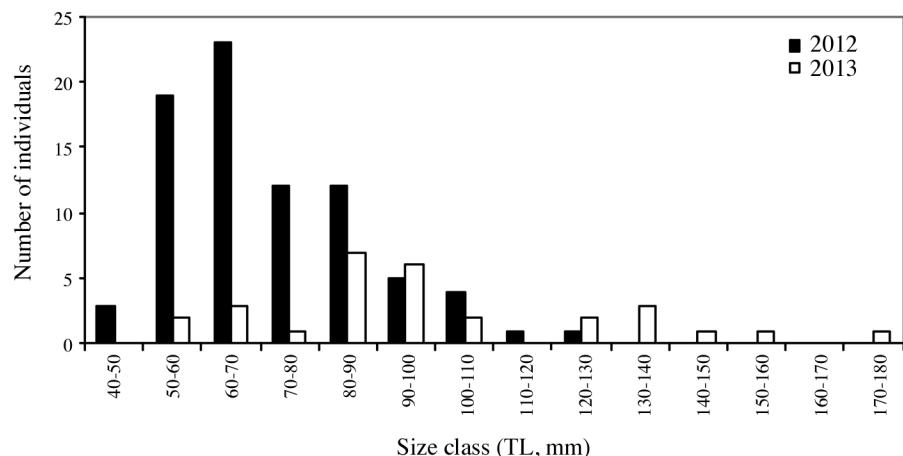


Figure 4. - Length-frequency distribution of juveniles *Hyporhamphus picarti* during autumn 2012 (black bar) and late summer 2013 (white bar).

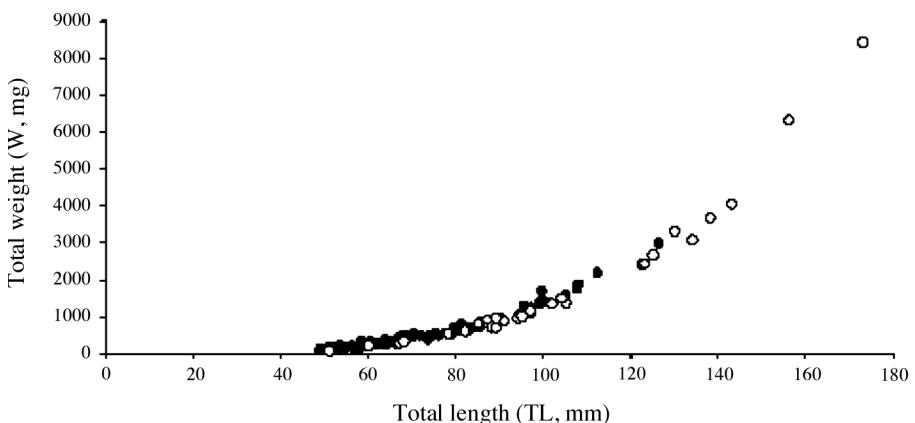


Figure 5. - Length-weight relationship of juveniles *Hyporhamphus picarti*. Black circles indicate individuals caught in autumn 2012, and white circles those caught in 2013. Equations are for 2012: $W = 0.0006 TL^{3.17}$ ($n = 52$; $r^2 = 0.96$; $p < 0.05$); and for 2013: $W = 0.0006 TL^{3.17}$ ($n = 29$; $r^2 = 0.71$; $p < 0.05$).

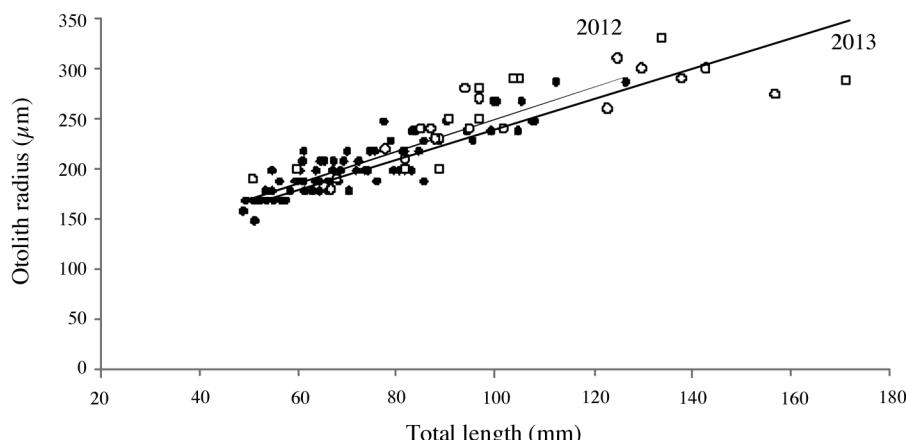


Figure 6. - Relationship between otolith (*lapillus*) radius (OR, μm) and total length (TL, mm) of juvenile *Hyporhamphus picarti*. Black circles indicate individuals caught in autumn 2012, and white circles those caught in 2013. Linear growth equations are for 2012: $\text{OR} = 1.622 \times \text{TL} + 88.79$ ($n = 52$; $r^2 = 0.80$; $p < 0.05$); and for 2013: $\text{OR} = 1.055 \times \text{TL} + 143.07$ ($n = 29$; $r^2 = 0.56$; $p < 0.05$).

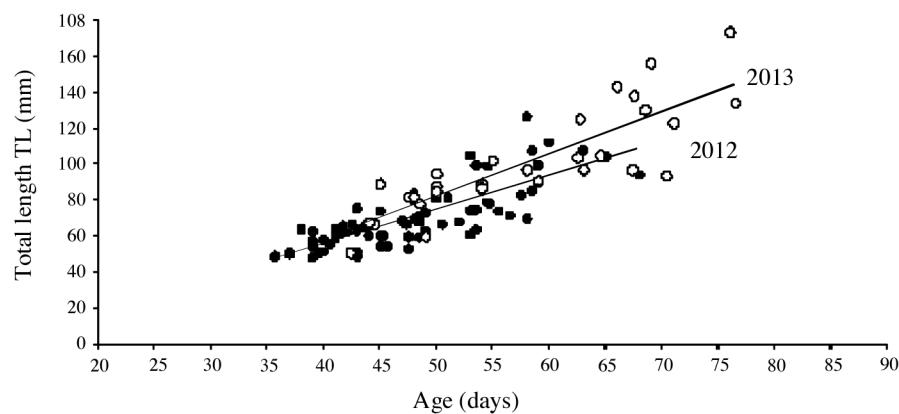


Figure 7. - Relationship between total length (TL, mm) and number of increments in otolith of juvenile *Hyporhamphus picarti*. Black circles indicate individuals caught in autumn 2012, and white circles those caught in 2013. Linear growth equations are for 2012: $\text{LT} = 1.881 \times \text{age} - 19.29$ ($n = 52$; $r^2 = 0.63$; $p < 0.05$); and for 2013: $\text{LT} = 2.353 \times \text{age} - 35.57$ ($n = 29$; $r^2 = 0.71$; $p < 0.05$).

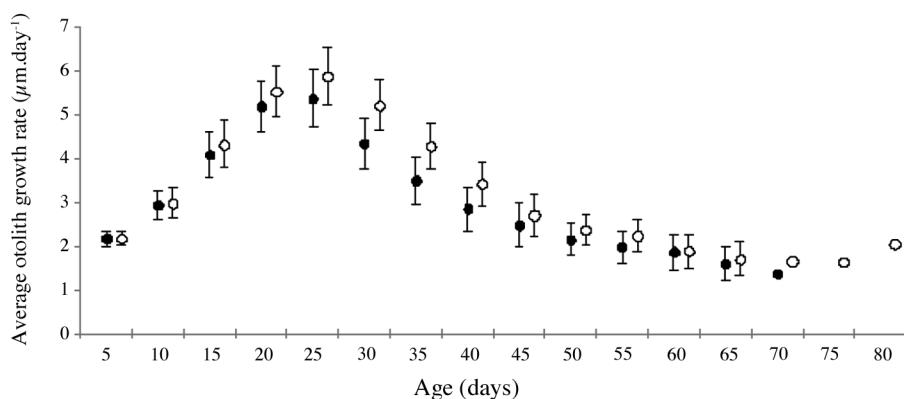


Figure 8. - Average otolith growth rate ($\mu\text{m}/\text{day}$) of juvenile *Hyporhamphus picarti* (means \pm SD) measured in the postrostrum axis from core. Black circles indicate individuals caught in autumn 2012, and white circles those caught in 2013.

DISCUSSION

The African halfbeak *H. picarti* is described for the first time in the Nador lagoon. This species was observed only in lagoons located in the SW part of the Mediterranean, in Ichkeul in Tunisia (Kraiem *et al.*, 2003), and recently in the Mellah lagoon in Algeria (Embarek, unpubl. data). Although lagoons located in the northern part of the Mediterranean have been intensively studied, this species was never recorded. Examples include: Venice lagoon (Franco

et al., 2006); Lake Fogliano and Caprolace lagoons in Italy (Mariani, 2001); Vaccares lagoon in France (Poizat *et al.*, 2004); Corsica lagoons (Bouchoucha, 2010); Mar Menor in Spain (Pérez-Ruzafa *et al.*, 2004); and Porto-Lagos Lagoon in Greece (Koutrakis *et al.*, 2005). The species has only been described in the Guadalquivir Estuary (SW Spain) (Sobrino *et al.*, 2005). The Nador lagoon serves as nursery ground for juvenile *H. picarti* only during late summer and autumn. The seine net used was considered to be appropriate for catching juvenile (< 100 mm TL) fish in shallow waters, especial-

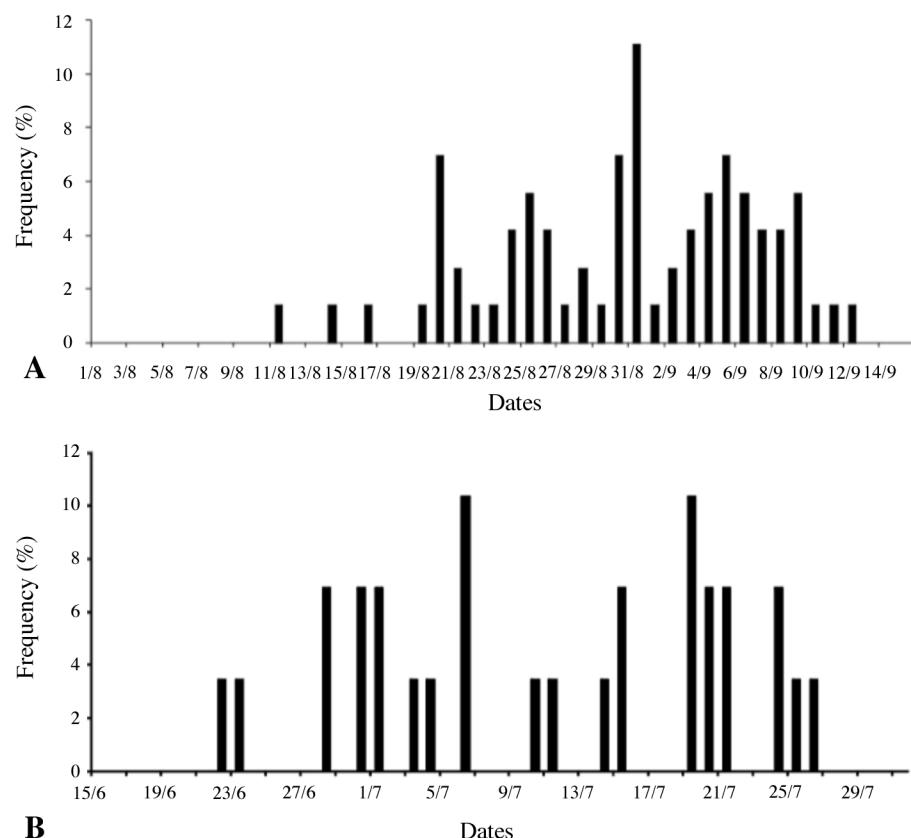


Figure 9. - Hatch-date frequency distribution of juvenile *Hyporhamphus picarti* caught during A: late summer 2012, and B: early autumn 2013.

ly in lagoon ecosystems (Pierce *et al.*, 1990; Franco *et al.*, 2012). The smallest individual caught in this study measured 38 mm TL, which corresponds to an age of about 31 days, suggesting that this species colonizes the lagoon at late larval or early juvenile stages. During late summer and autumn, *H. picarti* forms an important part of the fish assemblage of the lagoon. The majority of the specimens was caught near or in the plant-covered areas of the lagoon (bottom covered by seagrass meadows, mainly *Cymodocea*). *H. picarti* is associated with seagrass beds in every region of the world where it is found. These plants can be used as a support for eggs after spawning, and are one of the basic elements of their diet (Noell, 2005; Tibbetts *et al.*, 2008; Earl *et al.*, 2011).

This study estimated the age, growth, and hatching date period of *H. picarti* for the first time. Few estimates of growth of early life stages of Hemiramphidae are available in the literature. The discovery of growth increments in the microstructure of otolith (Pannella, 1971) has led to a more accurate estimation of the age and growth of larval and juvenile fish. Ageing by counting otolith daily growth increments provides a means to estimate growth and back-calculate hatching dates (Campana and Jones, 1992). Although the periodicity of increment formation and the timing of the first increment formation were not yet validated for *H. picarti*, numerous studies have demonstrated that such increments

form daily in a wide variety of fish species (see reviews by Campana and Neilson, 1985; Jones, 1986). Such validation has been done for some species of the genus *Hyporhamphus* such as *H. sajori* (Nakaya *et al.*, 2008) and *H. unifasciatus* (Monteiro, 2003). We therefore assume the daily nature of the growth increments of *H. picarti* otolith. The increased use of lapillar otoliths of late larvae or juvenile fish for increment counts (e.g. Amara *et al.*, 1994; Hernández *et al.*, 2015) generally results from the fact that the lapillar otoliths are relatively thin and that the increments are well-defined and spatially more uniform.

In this study, the otolith average increment widths varied from 2 to 6 μm , and were well above the visual threshold of equipment generally employed, since the limit of resolution of light microscopy is theoretically c. 0.2 μm (Campana and Neilson, 1985). A strong linear relationship between fish size (TL, mm) and otolith radius was found, indicating that somatic growth history can be reconstructed from otolith growth patterns. These results showed that for both years, the average width of daily increment increased until about the 25th day (corresponding to a size of about 28 mm TL), and then decreased. This slowdown in otolith growth may have corresponded to an ontogenetic change, such as the metamorphosis period observed in Japanese halfbeaks, *Hyporhamphus sajori* (Futami *et al.*, 2002). After this period, increment

widths were significantly higher in 2013 compared to 2012, suggesting a higher juvenile growth during 2013. Indeed, the instantaneous growth rate calculated from the growth curve was significantly higher in 2013. Although growth studies on Hemiramphidae species, including early juvenile individuals, are scarce in the literature, the growth rates estimated in the present study (1.88 mm.d^{-1} and 2.35 mm.d^{-1} , respectively in 2012 and 2013) are comparable to those estimated for *H. brasiliensis*, 1.2 mm.d^{-1} (Berkeley and Houde, 1978) and 1.09 mm. d^{-1} for *H. melanochir* (Monteiro, 2003). Halfbeak species are known to grow rapidly and mature during their first year (McBride and Thurman, 2003; Collette, 2004).

In this study, juvenile *H. picarti* captured in late summer and autumn in the Nador lagoon hatched in summer from June to September. The presence of juvenile individuals only in late summer and autumn suggests that this species has a short spawning period. *Hemiramphus* species such as *H. brasiliensis* and *H. balao* in the coastal waters of southeastern Florida spawn frequently, even daily, for at least a few months of the year (Mc Bride and Thurman, 2003). However, prolonged reproductive season have been noted for many other hemiramphids (e.g. *H. melanochir* (Ling, 1958; Jones et al., 2002), *Zenarchopterus kampeni* (Coates and van Zwieten, 1992)). In the coastal waters of New South Wales, Australia, the reproductive activity of eastern-sea garfish *H. australis* and of eastern river garfish *H. regularis ardelio* occurs during a seven-month period between June and December (Hughes and Stewart, 2006). Spawning of Hemiramphidae often occurs during the warmer months of the year (Jones et al., 2002; McBride and Thurman, 2003; McBride et al., 2003). The spawning cycles of temperate marine fish are often linked to environmental cues, most commonly day length, temperature, or both (Bye, 1990). Studies on the hemiramphids have also suggested that spawning coincides with high abundances of zooplankton, which is an important larval food source (Jones et al., 2002). In the Nador lagoon and adjacent coastal areas, higher temperature, and plankton abundance occur during the summer months (El Madani et al., 2011; Zaafa et al., 2012). Such conditions enhance *H. picarti* reproduction, and larval development and survival. The difference in temperature during the two years of study does not explain the observed variation in growth and hatching dates. Others environmental parameters such as food abundance may have more influence on the early population dynamic of this species.

CONCLUSIONS

This study on the African halfbeak *H. picarti* provides, for the first time, biological and ecological information, which may be useful for its management and conservation. The Nador lagoon is used as nursery ground for juvenile

H. picarti only during the late summer and autumn, when the species constitutes an important part of the fish assemblage in the lagoon. Back-calculated hatching dates of juveniles have indicated that they hatch in summer from June to September, and suggest that *H. picarti* has a short spawning period. A growth change was observed around the 25th day post hatching and probably corresponds to metamorphosis or habitat change (lagoon colonization).

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